

Sex expression and reproduction of four bryophytes following timber harvesting

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Abstract

Many factors are reported to affect reproduction in bryophytes but studies on the response of sexual reproduction to logging are extremely rare and the authors know of none for Australia. This paper reports the seasonal changes in sex expression and phenological development across a post-logging chronosequence for four species. Three species, *Rhaphidorrhynchium amoenum*, *Rhynchostegium tenuifolium* and *Wijkia extenuata*, were pleurocarpous and one, *Rosulabryum billardieri*, was an acrocarpous species. Reproduction in pleurocarpous species is seldom investigated because of the difficulty of untangling individual stems. Sex expression in terms of the numbers of male and female stems, number of perichaetia and perigonia, number of archegonia, atheridia and sporophytes did not differ across the chronosequence, so it would appear that forest age did not affect reproductive phenology. (*The Victorian Naturalist*, 132 (6), 2015, 160-169)

Keywords: bryophytes, phenology, reproduction, logging, chronosequence

Introduction

It is essential that bryophytes have the ability to reproduce sexually for harvested forests to return to pre-harvest levels of bryophyte composition and richness with a high degree of genetic diversity amongst populations. Many bryophytes reproduce only by asexual means (Longton 1976), implying they may be unable to cope with changing environments (Stark *et al.* 1998); however, as long as periods of sexuality occur, there is no reason why bryophytes could not benefit from both long- and short-term advantages provided by both forms of reproduction (Longton 1976). Asexual reproduction is considered important for colony expansion and maintenance (Longton 1976, 2006), while sexual reproduction is considered important for genetic diversity, providing for the potential of survival in changed conditions and development of new populations in a new ecology (Longton 2006).

Many factors affect reproduction in bryophytes. For example, stress caused by changes in the physicochemical nature of the environment is known to promote a female-biased sex ratio (Stark 2002). Bryophytes depend on the appropriate signals for sex expression, and developmental pattern of gametangia and sporophytes. If the appropriate signals are lacking, the phenological events may not occur. Inhibition of archegonial maturation, when sperm are ready for release, would prevent fertilisa-

tion events (Longton 1972). If conditions have changed, growth can be affected. This, in turn, can affect reproduction. Benassi *et al.* (2011) concluded that limited water availability could stunt plants and thus inhibit sex expression, promote growth of female-only individuals and, therefore, limit sexual reproduction. Their data suggested that males of *Syntrichia caninervis* required more consistent water availability as they had a lower tolerance for repeated cycles of wetting and drying. Younger forests would have very different wetting and drying cycles from those in older forests, which, therefore, could affect reproduction. Needless to say, other factors that could be important to reproduction also vary between younger and older forests. Photoperiod would be shorter in a more closed forest than open forest because of shading effects. Similarly, temperatures are more extreme in open than closed forests. Nutrient levels also vary with forest age and may affect reproduction. The high nutrient demand necessary for reproduction means that limited nutrient levels can inhibit reproduction. In *Syntrichia caninervis*, Bowker *et al.* (2000) found greater sex expression was associated with shady microhabitats, higher soil moisture, greater nutrient availability and taller ramets. Male ramets were restricted to shaded microhabitats whilst female ramets were found in both shaded and exposed microhabitats. Forests at various stages

of regeneration post-logging vary markedly in moisture levels, light and temperature regimes and nutrient availability, thus cues for sexual reproduction may vary with time or, in fact, be absent. It is important, thus, to understand logging effects on bryophyte reproduction.

Such studies are extremely few and, currently, effects of logging on bryophyte reproduction can be inferred only from studies where another disturbance has been investigated; however, Cronberg *et al.* (2003) investigated the sex ratio in the moss *Plagiommium affine* in forests of contrasting age after timber harvest, screening for genetic variation at 23 allozyme loci. They found female bias occurred at the ramet level but balanced sex ratios occurred at the genet level. Forest age was positively correlated with sporophyte numbers and negatively correlated to the percentage of non-expressed shoots.

The number of studies on sex ratios outside the logging scenario is considerable. Such studies involve investigation into the number of stems bearing perichaetia or perigonia and whether sexual reproduction is taking place, usually evidenced by the presence and number of sporophytes. The number of non-expressed stems also is taken into account. Given that 60% of bryophytes worldwide are dioicous (Wyatt and Anderson 1984), most studies on sex ratios have been undertaken on dioicous species, whilst monoicous species have been neglected. Dioicous species are known for having a female biased sex ratio and high levels of non-expressed stems, with many studies on individual species showing this (Shaw and Gaughan 1993; Stark *et al.* 1998; Benassi *et al.* 2011; Cronberg *et al.* 2003; Stark *et al.* 2010; Rydgren *et al.* 2010). Bisang and Hedenäs (2005) reviewed the sex ratios of 89 dioicous moss and liverwort species using literature reports and their own investigations, as well as herbarium specimens, of which ten were from Australia. They found 88% of herbarium specimens or 'patch in the field' and 68% of 'shoots in the field' showed a female skewed ratio. This 'trait' of dioicous species is strange, given that sex chromosome formation through meiosis 'should' result in the formation of male and female spores in equal numbers (Shaw and Gaughan 1993; Bisang and Hedenäs 2005). A number of possible explanations have been put forward to explain this phe-

nomenon. Stark (2002) suggested that female skewed sex ratios may be a product of a higher realised cost of sexual reproduction in males; however, Bisang *et al.* (2006) found *Pseudocalliergon trifarium* showed no detectable costs to explain male rarity when they investigated the cost of allocation to sexual branches.

Monoicous bryophytes develop both male and female gametangia on the one stem and have been found to be self-fertilising in the mosses *Phascum cuspidatum*, *Pottia truncata* and *Weissia controversa* (Roads and Longton 2003). Cross fertilisation takes place in other monoicous species such as *Atrichum undulatum*, *Tortula muralis* (Longton and Miles 1982) and *Entodon cladorrhizans* (Stark 1983). The ability to self-fertilise produces a higher number of sporophytes in monoicous as opposed to dioicous species.

This study investigated the sex expression of four bryophyte species inhabiting forests regenerating after clearfell-burn-sow logging over a chronosequence in Wet Sclerophyll Forest (WSF) in the state of Victoria, Australia. The specific aims were to determine: (1) if sexual reproduction occurred; (2) the timing of phenophases and whether these were affected by years-since-harvest; (3) whether a bias in sex expression occurred; and (4) if sex expression altered with years-since-harvest.

Methods

Study Area

Toolangi State Forest is located in the Central Highlands of Victoria approximately 80 km north-east of Melbourne (Fig. 1). The climate is described as temperate with a mean annual temperature of 15.8°C and monthly means ranging from 8.6°C to 23.2°C. The mean annual rainfall is 1370 mm. Mean monthly rainfall varies from 77 mm to 138.5 mm (Bureau of Meteorology 2008).

The area has wet sclerophyll forest dominated by *Eucalyptus regnans*, which is able to reach heights of 100 m (Attiwill and May 2001; Costermans 1996) although records prior to 1935 included specimens higher than 100 m (Ashton 1975; 2000; Beadle 1981; Hardy 1968; DNRE 1996). *Eucalyptus regnans* is the tallest (Beadle 1981) and fastest growing eucalypt (Ashton and Attiwill 1994), thus such ecosystems are used



Fig. 1. Location of the Central Highlands (green) in Victoria, Australia.

widely for forest harvesting. Selective logging was undertaken in Toolangi State Forest prior to severe bushfires in 1926 and 1939, and salvage operations were conducted following these fires (Ough and Ross 1992; DNRE 1996). Since the 1960s, clearfell logging has been the major silvicultural technique used in the area.

Sampling

Forests of five age classes along a chronosequence were selected for investigation, all previously having undergone clearfell logging. Applicable sites were derived from logging history maps supplied by the then Department of Natural Resources and Environment. Sites consisted of areas logged 10, 15, 20, 25 and 30 years prior to the study. All had been burnt previously in the 1939 wildfire. These ages were chosen because the target species occurred frequently, allowing for the sampling regime. When these species were present within younger sites, they occurred less frequently, more sporadically, and in much smaller populations. Also investigated was a 63-year-old forest regenerating from the 1939 wildfire that had not been logged or burnt since.

A 900 m² quadrat was examined within each of five sites from each age class. Sites were determined using a computer-generated random number table. Quadrat size and number was determined by the use of a 'species area curve' (Andrew and Mapstone 1987) undertaken in the 63-year-old forest, which was visually most species-rich.

To limit any possible edge effects, quadrat placement was at least 50 m from any road edge or forest of a different age or type. Sampling occurred from October 2002 to November 2003.

Four bryophyte species common to wet sclerophyll forests were chosen for investigation: *Wijkia extenuata* (Fig. 2a), *Rhaphidorrhynchium amoenum* (Fig. 2b), *Rosulabryum billardierii* (Fig. 2c) and *Rhynchostegium tenuifolium* (Fig. 2d). *Rhaphidorrhynchium amoenum* is a monoicous species widespread throughout Australia and New Zealand (Scott and Stone 1976). It is found in all but the driest of habitats (Meagher and Fuhrer 2003) on trunks of trees, rocks, soil and logs. It grows in densely woven mats and is pinnately branched. *Rhynchostegium tenuifolium* is a soft, slender, pleurocarpous moss quite variable in its appearance, either matted into flattened tufts or loose and straggly. It is an autoicous species, which means the perichaetia and perigonia occur on the same plant, but never on the same stem or branch. It is found widely throughout southern Australia (Meagher and Fuhrer 2003), and commonly inhabits soil, logs and bark in wet habitats. *Rosulabryum billardierii* is an acrocarpous, dioicous species widely spread throughout all of Australia. It occurs also in Asia, South and Central America, Africa, New Zealand, Oceania and Europe (Scott and Stone 1976). It is found in many habitats but is known to occur mostly in wet environments. *Wijkia extenuata* is also a dioicous species common to wet habitats throughout Victoria, Tasmania, ACT and NSW (Scott and Stone 1976). It also occurs in New Zealand. It is a pleurocarpous, prostrate, matted moss, commonly found on logs, soil, trunks, rocks and ferns.

Within each forest of differing age, 50 stems of each species were collected seasonally over a 12 month period. Each stem was examined for the presence of perichaetia, perigonia and sporophytes, the number of each was recorded and sex expression of stems determined. They were further examined for the number of antheridia and archegonia. These, along with the sporophytes, were assigned a maturation stage (Longton and Green 1969) (Table 1).

Data Analysis

Analysis of similarity (ANOSIM) was conducted to determine if a difference in the num-

Table 1. Maturation stages of mosses (derived from Longton and Greene 1969)

Stage	Description
Sporophytes	
Swollen venter (SV)	Venter of archegonium begins to swell
Early calyptra in perichaetium (ECP)	Calyptra assumes pale yellow colour
Late calyptra in perichaetium (LCP)	Calyptra becomes half exerted from perichaetial bracts
Early calyptra intact (ECI)	Calyptra becomes fully exerted from perichaetial bracts
Late calyptra intact (LCI)	Swelling of capsule begins
Early operculum intact (EOI)	Operculum becomes brown in colour
Late operculum intact (LOI)	Capsule becomes brown in colour
Operculum fallen (OF)	Operculum falls
Empty and fresh (EF)	75% of spores are shed
Aborted (A)	Apex of sporophyte wither prior to spore formation, usually in ECP, LCP or ECI stages
Gametangia	
Juvenile (J)	Gametangia become visible. Pale green colour
Immature (I)	Gametangia reach half length of dehiscent gametangia
Mature (M)	Apices of gametangia rupture. Archegonia become receptive for fertilisation and liberation of antherozoids begins.
Dehiscent (D)	Development of brown colouration begins in gametangia at ruptured apices
Aborted (A)	Development of brown or hyaline colouration begins in gametangia with unruptured apices in J or I stages



Fig. 2. a) *Wijkia extenuata*; b) *Rhabdorrhynchium amoenum*; c) *Rosulabryum billardieri*; and d) *Rhynchosyium tenuifolium*. Photos by Bernadette Sinclair.

ber of perichaetia, perigonia, sporophytes and gametangia existed for each species across the chronosequence. ANOSIM provides a test statistic, R , between -1 and 1 . If $R=0$, there is no difference in the reproductive traits along the chronosequence. If $R=1$ or -1 , perfect separation exists.

Results

Sex expression

Sex expression of stems within each forest age-class was higher than non-expression for the three pleurocarpous species (Table 2). The two dioicous species, *Rosulabryum billardieri* (acrocarpous) and *W. extenuata*, produced more female than male stems (Table 2b, d). The female bias was very strong in *Rosulabryum billardieri*, as few male stems occurred; however, the total number of sexually non-expressing stems was high for this species. At times the number of perigonia or antheridia occurring within an age class reduced the female bias, especially in *W. extenuata*. In *W. extenuata*, there was a trend for variation in sex expression of stems to be greater in forests at either end of the temporal chronosequence. In no instance did statistically significant variation of reproductive attributes occur across the temporal chronosequence for either species (*Rosulabryum billardieri*: Fertile and non-fertile stems: Global $R=0.03$; $p=0.63$. Perichaetia and perigonia: Global $R=0.08$; $p=0.81$. Archegonia and antheridia: Global $R=0.53$; $p=0.06$. *W. extenuata*: Fertile and non-fertile stems: Global $R=0.06$; $p=0.70$. Perichaetia and perigonia: Global $R=0.01$; $p=0.56$. Archegonia and antheridia: Global $R=0.13$; $p=0.94$).

Rhaphidorrhynchium amoenum is recognised as a monoicous species (Scott and Stone 1976) and although the majority of stems expressed monoicy, a number of stems were found to bear solely female or solely male organs (Table 2a). The female bias was clearly shown in terms of higher numbers of perichaetia although greater numbers of antheridia than archegonia occurred within four age classes. Forest age did not show an affect on any of these reproductive attributes (Fertile and non-fertile stems: Global $R=0.17$; $p=0.03$. Perichaetia and perigonia: Global $R=0.11$; $p=0.92$. Archegonia and antheridia: Global $R=0.43$; $p=0.99$). Pairwise tests showed

a significant difference occurred between forests of 15 and 25 years-since-harvest ($R=0.698$; $p=0.03$) but this one-off occurrence was not considered indicative of an effect of forest age and is attributed to chance.

Rhynchostegium tenuifolium did not bear any female only or male only stems; all stems bore both sexes as expected for a monoicous species (Table 2c). Perigonia outnumbered perichaetia in forests of 15–30 years-since-harvest, as did antheridia compared to archegonia, thus a male bias occurred. Antheridia also outnumbered archegonia in forests of 10 years-since-harvest. Again, forest age did not show any effect on reproductive attributes (Fertile and non-fertile stems: Global $R=0.10$; $p=0.86$. Perichaetia and perigonia: Global $R=0.13$; $p=0.04$. Archegonia and antheridia: Global $R=0.15$; $p=0.90$).

Only *Rhaphidorrhynchium amoenum* showed a comparatively respectable number of sporophytes (795) from a total of 1200 stems (Table 2a). The other three species had 151 or fewer sporophytes out of 1000 stems (Table 2b-d). ANOSIM showed no significant differences across the temporal chronosequence for any of the four moss species (*Rhaphidorrhynchium amoenum*: Global $R=0.13$; $p=0.95$. *Rhynchostegium tenuifolium*: Global $R=0.23$; $p=0.93$. *W. extenuata*: Global $R=0.18$; $p=0.99$. *Rosulabryum billardieri*: Global $R=0.25$; $p=0.19$).

Phenology

Generally, the sequence and timing of sporophyte development for each species was similar for each forest age class so data was pooled for each species and peaks of each phenostage were used to better show the phenological development (Fig. 3). *Rhaphidorrhynchium amoenum* began sporophyte development in spring with the production of swollen venters, while *W. extenuata* began in summer. Both showed a similar sequence of development, and completed their cycles within 12 to 14 months with empty and fresh sporangia peaking in summer (Fig. 3). The data for *Rhynchostegium tenuifolium* have gaps but are suggestive of a similar developmental sequence to those of *Rhaphidorrhynchium amoenum* and *W. extenuata* (Fig. 3). Data for *Rosulabryum billardieri* were insufficient to make any meaningful deductions as to the developmental sequence of sporophytes.

Table 2. Sex expression of a. *Rhaphidorrhynchium amoenum* b. *Rosulabryum billarderii* c. *Rhynchostegium tenuifolium* d. *Wijkia extenuata* in different aged forest post harvest (NE = Non expressed stems).

a. *Rhaphidorrhynchium amoenum* (pleurocarpous)

Number of:	Forest age						Total	Mean
	10	15	20	25	30	63		
Stems examined	200	200	200	200	200	200	1200	
% fertile stems	65.5	82.5	75.5	55.5	70.5	53.5		67.2
% NE stems	34.5	17.5	24.5	44.5	29.5	46.5		33
Female stems	0	6	12	5	0	0	23	
Male stems	0	9	6	5	0	0	20	
Male & female stems	131	150	133	101	141	107	763	
NE stems	69	35	49	89	59	93	394	
Perichaetia	304	223	236	205	243	274	1485	
Perigonia	166	197	229	187	264	150	1193	
Ratio perichaetia:perigonia	1.8:1	1.13:1	1.03:1	1.09:1	1:1.09	1.8:1		1.2:1
Archegonia	93	138	75	224	0	123	653	
Antheridia	285	91	338	72	190	215	1191	
Ratio archegonia:antheridia	1.3:1	1.5:1	1:4.5	3.1:1	0:1	1:1.8		1:1.8
Sporophytes	163	153	112	123	113	130	794	
Fertile stems with sporophytes	131	165	151	111	141	107	806	

b. *Rosulabryum billarderii* (acrocarpous)

Number of:	Forest age					Total	Mean
	10	15	20	30	63		
Stems examined	200	200	200	200	200	1000	
% fertile stems	23.5	15	35.5	28	31		26.6
% NE stems	76.5	85	64.5	72	69		73.4
Female stems	46	29	58	49	53	235	
Male stems	1	1	13	7	9	31	
NE stems	153	170	129	144	138	734	
Perichaetia	46	29	58	49	53	235	
Perigonia	1	1	13	14	9	38	
Ratio perichaetia:perigonia	46:1	29:1	4.5:1	3.5:1	5.9:1		6.2:1
Archegonia	25	168	0	54	45	292	
Antheridia	14	0	87	24	0	125	
Ratio archegonia:antheridia	1.8:1	168:0	0:9	2.3:1	45:0		2.3:1
Sporophytes	11	7	12	10	20	60	
Fertile stems with sporophytes	47	30	71	56	62	266	

c. *Rhynchostegium tenuifolium* (pleurocarpous)

Number of:	Forest age					Total	Mean
	10	15	20	25	30		
Stems examined	200	200	200	200	200	1000	
% fertile stems	48.5	37.5	46.5	35	41.5		41.8
% NE stems	51.5	62.5	53.5	65	58.5		58.3
Male & female stems	97	75	93	70	82	417	
NE stems	103	125	107	130	118	583	
Perichaetia	120	84	94	134	128	560	
Perigonia	103	127	104	188	95	617	
Ratio perichaetia:perigonia	1.2:1	1:1.5	1:1.1	1:1.4	1.4:1		1:1.0
Archegonia	186	84	102	78	88	538	
Antheridia	311	323	300	442	78	1454	
Ratio archegonia:antheridia	1:1.7	1:3.9	1:2.9	1:5.7	1:1.1		1:2.7
Sporophytes	12	5	17	23	33	90	
Fertile stems with sporophytes	97	75	93	70	82	417	

Table 2 cont.

d. *Wijkia extenuata* (pleurocarpous)

Number of:	Forest age						Total	Mean
	10	15	20	25	30	63		
Stems examined	200	200	200	200	200	200	1200	
% fertile stems	59.5	44.5	58.5	69	60	69.5		60.2
% NE stems	40.5	55.5	41.5	31	40	30.5		41.3
Female stems	71	38	62	70	61	83	385	
Male stems	30	51	55	68	59	56	319	
Ratio female:male stems	2.4:1	1:1.3	1.1:1	1.0:1	1.0:1	1.5:1		1.2:1
NE stems	99	111	83	62	80	61	496	
Perichaetia	129	77	146	118	106	169	745	
Perigonia	94	229	204	186	225	357	1295	
Ratio perichaetia:perigonia	1.37:1	1:2.97	1:1.4	1:1.58	1:2.12	1:2.11		1:1.7
Archegonia	141	142	142	124	99	170	818	
Antheridia	131	439	355	147	115	451	1638	
Ratio archegonia:antheridia	1.08:1	1:3.09	1:2.5	1:1.19	1:1.16	1:2.65		1:2.0
Sporophytes	30	26	24	17	21	33	151	
Fertile stems with sporophytes	119	89	117	138	120	139	722	

Age of forest did not seem to have affected the timing and sequence of development for archegonia and antheridia so, as was done for sporophytes, data were pooled for each species to better demonstrate any pattern in phenological development. All stages of development in *Rhaphidorrhynchium amoenum* occurred in spring although dehisced archegonia were also noted in summer and winter. The observation of mature archegonia receptive for fertilisation during spring supports the findings that the number of sporophytes at the swollen venter stage occurred during this season (Fig. 3). Juvenile and immature antheridia peaked in winter while mature and dehisced antheridia peaked in spring, suggesting antherozoids were available for fertilisation. The maturation of the archegonia and antheridia and the occurrence of swollen venters during spring provide strong evidence that fertilisation occurs during this season.

The number of juvenile archegonia of *W. extenuata* peaked in autumn, which was followed by a peak in number of immature archegonia in winter, then a peak in number of mature archegonia in winter and spring and, lastly, a peak in number of dehisced archegonia in summer. The data for antheridia did not allow interpretation of a phenological sequence.

The juvenile, immature and mature phenostages of both archegonia and antheridia of *Rhynchostegium tenuifolium* peaked in summer

but dehisced stages peaked in autumn. The accompanying peak in swollen venters in summer (Fig. 3) strongly suggests fertilisation occurred during this season.

With respect to *Rosulabryum billardierii*, all phenostages of archegonia peaked in spring although juvenile and mature stages occurred in good numbers in winter, but only in forests of 15 years-since-harvest. Antheridia were noted only in spring but all were at the immature stage.

Discussion

Each of the four species produced sporophytes at the swollen venter stage when gametangia were mature and gametes were available for fertilisation events. Thus the evidence is very strong that sexual reproduction occurs in each of the four species examined and does so regularly, although the data for both *W. extenuata* and *Rosulabryum billardierii* were insufficient to determine the phenological development of antheridia.

Although the data sets were incomplete, as not all stages were found in all forest age groups, forest age did not appear to inhibit sexual reproduction or its timing. As logging was shown to reduce species richness significantly (Sinclair 2012), these findings are reassuring in that they suggest logging does not affect the sexual abilities and capacities of those species able to recolonise, and they should be able to survive in the regenerating forests. These four species, however, are common, and the story for less common

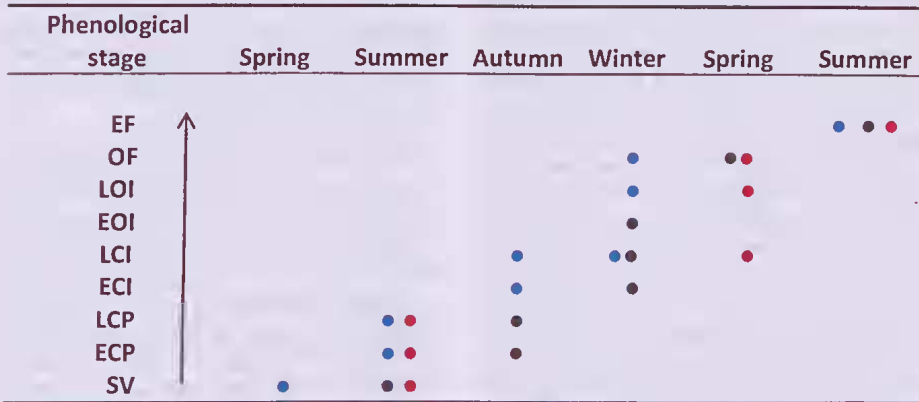


Fig. 3. Sporophyte developmental sequences for *Rhaphidorrhynchium amoenum* (blue), *Wijkia extenuata* (black) and *Rhynchostegium tenuifolium* (red) showing peaks of occurrence for phenological stages. Data was pooled from forest regenerating following logging (i.e. at 10, 15, 20, 25 and 30 years-since-harvest) and from a 63 year old forest. Arrow indicates increasing level of maturity. SV=Swollen venter, ECP=Early calyptra in perichaetium, LCP=Late calyptra in perichaetium, ECI=Early calyptra intact, LCI=Late calyptra intact, EOI=Early operculum intact, LOI=Late operculum intact, OF=Operculum fallen, EF=Empty and fresh.

species may be very different and require further investigation. Other studies, such as those of Cronberg *et al.* (2003), found logging did affect sexual reproduction. They examined the effects of forest age post-logging in *Plagiomnium affine* and found that female bias occurred at the ramet level although balanced sex ratios occurred at the genet level. They also found forest age positively correlated with sporophyte numbers and negatively correlated to the percentage of non-expressed shoots. Obviously, the results for one species cannot necessarily be used to predict what happens to another species, although in management this frequently occurs.

It was not surprising that logging did not affect sexual reproduction, as many mosses show strong seasonality in terms of reproductive development and fertilisation events. This suggests temperature, moisture levels and daylength beyond the range of variation caused by logging would be triggers for the onset of phenological events. This, in turn, suggests the seasonal cycle is genetically controlled (Mishler and Oliver 1991; Sinclair 1999). Seasonality of the phenological cycle has been demonstrated for *Atrichum androgynum* (Biggs and Gibson 2006), *Atrichum undulatum*, *Bryum argenteum* (Miles *et al.* 1989), *Atrichum angustatum* (Zehr 1979), *Mnium hornum* (Greene 1967), *Pleu-*

rozium schreberi (Longton and Greene 1969), *Dicranoloma billarderii*, *D. platycaulon* and *D. menziesii* (Milne 2001). In other species, the sporophytic cycle is seasonal although the gametangial cycle is not, e.g. *Grimmia pulvinata* and *Tortula muralis* (Miles *et al.* 1989). Other species, e.g. *Funaria hygrometrica*, show no seasonality in development of their phenostages but can produce gametangia and sporophytes throughout the year (Longton 1976).

Development of sporophytes in *Rhaphidorrhynchium amoenum*, *W. extenuata* and *Rhynchostegium tenuifolium* extended over a 12 to 14 month period. This was not unusual. Some species complete development in a few months while others take years. *Dicranoloma platycaulon* and *D. billarderii* take 18 to 24 months (Milne 2001), *Atrichum rhystophyllum*, *Pogonatum inflexum* (Imura 1994) and *Entodon cladorrhizans* (Stark 1985) take nine months, and *F. hygrometrica* can take as little as two months (pers. obs. M Gibson).

Archegonia often undergo rapid development while antheridial development often requires more time (Imura 1994; Miles *et al.* 1989; Milne 2001). In these situations, maturation of both the male and female gametangia often occur at the same time and facilitates more successful fertilisation, particularly in dioicous spe-

cies (Longton and Greene 1967, 1969; Imura and Iwatsuki 1989). This study examined two dioicous, *Rosulabryum billardieri* and *W. extenuata*, and two monoicous species, *Rhaphidorrhynchium amoenum* and *Rhynchostegium tenuifolium*. The length of time required for development of the gametangia of the four species examined in this study requires clarification.

Sixty per cent of bryophytes are considered dioicous (Wyatt and Anderson 1984). Thus it is understandable that most published studies concerning sex ratios were on dioicous species. Dioicous species are known for their female-biased sex ratios and high levels of non-expressed stems; however, while *Rosulabryum billardieri* continued the trend with a higher level of non-expressed stems than fertile stems, *W. extenuata* had a greater number of stems showing sex expression. If stress had an effect on the number of female and male stems produced, it would be expected to be seen in species of the younger, more open forest, where the moss is more exposed and subjected to less humidity and shade than in the older forests of 30 and 63 years-since-harvest. This, however, was not the case; neither species showed a significant difference in sex ratio with forest age in terms of either stem number or the number of inflorescences.

The monoicous mosses, *Rhaphidorrhynchium amoenum* and *Rhynchostegium tenuifolium*, were expected to have high numbers of sporophytes due to the ability of many monoicous species to self-fertilise. This was found to occur in Japan where investigation into 81 mosses, 61 dioicous and 20 monoicous, found the monoicous mosses had much higher rates of fertilisation than the dioicous species (Une *et al.* 1983 as cited in Stark 2002). In this study, whilst *Rhaphidorrhynchium amoenum* followed suit with sporophytes on 98% of fertile stems, *Rhynchostegium tenuifolium* had sporophytes on only 21% of fertile stems. Kimmerer (1991) found that in the monoicous species *Tetraphis pellucida* sex expression increased as shoot density increased. This phenomenon was not examined in this study. Kimmerer also found that as shoot density increased, the number of males increased and grew to outnumber the females. Both species showed a tendency towards a male bias although this was not statistically significant. *Rhynchostegium tenuifolium* dem-

onstrated a male bias in terms of both perigonia and antheridia. *Rhaphidorrhynchium amoenum* had a greater number of antheridia than archegonia, which offset the greater number of perichaetia than perigonia.

Rhynchostegium tenuifolium has both male and female inflorescences on the same stem but on separate branches. This may contribute to the lower sporophyte production in *Rhynchostegium tenuifolium* compared to *Rhaphidorrhynchium amoenum*, which has both male and female inflorescences on the same stem or branch. Distance for sperm to travel would, therefore, be smaller in *Rhaphidorrhynchium amoenum*, thus fertilisation is likely to occur more often.

Concluding remarks

No statistically significant differences occurred in the number of unisexual or bisexual stems, or the number of inflorescences, gametangia or sporophytes across the chronosequence for any of the four species. Nor was there any obvious difference in timing of the reproductive phenostages for either sporophytes or gametophytes. This suggests that time post-harvest did not have a deleterious effect on the sex expression of mosses investigated. Other studies, however, have found effects of harvesting on bryophyte phenology and reproduction, or environmental effects that could be due to the changed conditions of forests regenerating subsequent to harvest. It is not possible, therefore, to use one species as a surrogate to predict what might occur in other species, as is often done in management. More studies on reproduction for Australian bryophyte species are recommended to provide for more informed management of bryophytes both within forests that are harvested and within our protected forests. Data used in this study were seasonal, and monthly sampling is recommended as it might better indicate any post-harvesting effects; however, this is very time-consuming.

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Glossary

(after Meagher and Fuhrer 2003)

- acrocarpous** Having sporophytes terminal on stems or branches. Old sporophytes can seem to be lateral as the branches resume growth in the subsequent growing season. The majority of acrocarpous mosses are erect.
- antheridium** (pl. antheridia) The male sex organ containing motile male gametes.
- antherozoid** Motile male gamete produced in the antheridium.
- archegonium** (pl. archegonia) The female sex organ containing the female gamete (ovum).
- calyptra** In mosses, a thin membrane protecting the developing sporophyte and forming a hood over the sporangium (structure containing spores). It is shed at maturity.
- dioicous** Having the antheridia and archegonia on different plants.
- inflorescence** Cluster of sex organs and the specialised leaves that surround them.
- monoicous** Having male and female sex organs on the same plant.
- operculum** The cap or lid covering the mouth of the capsule, which detaches at maturity to allow dispersal of the spores.
- perichaetium** (pl. perichaetia) Female inflorescence, comprised of specialised leaves surrounding the archegonia.
- perigonium** (pl. perigonia) Male inflorescence, comprised of specialised leaves surrounding the antheridia.
- pleurocarpous** Having the sporophytes arising from specialised side branches, so that the habit or form of the plant tends to be creeping or pendent.
- phenostage** Developmental stage that can be defined by a start and end point.
- venter** Swollen part of the archegonium, containing the egg.



Archegonia, each with a swollen venter at the base containing an ovum. Photo by Maria Gibson.